Beta-Amylase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6633

Detection and Quantification of Beta-Amylase Activity in Tissue extracts, Cell lysate Samples.

For research use only . Not for diagnostic or therapeutic procedures .

1. Introduction

Amylase belongs to the family of glycoside hydrolase enzymes that break down starch into glucose molecules by acting on α -1,4-glycosidic bonds. β -Amylase plays a central role in the complete degradation of starch to metabolisable or fermentable sugars during the germination or malting of cereal grains. It also finds considerable application, together with starch debranching enzyme s, in the production of high maltose syrups. β -Amylase is usually measured using non-specific reducing sugar assays with starch as substrate.

Beta-Amylase Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring Beta-Amylase activity in a variety of samples. Amylolytic enzyme hydrolyzes the starch to generate reducing sugar. The reducing sugar reduces the 3,5 -dinitrosalicylic acid to generate red-brown substance. The color intensity, measured at 540 nm, is proportionate to the enzyme activity in the sample.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	5 mlx 1	4 °C
Substrate	Powderx 1	4 °C
Dye Reagent	10 mL x 1	4 °C
Standard	Powderx 1	4 °C
Positive Control	Powderx 1	-20 °C
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water to generate 20 mmol/L of standard stock solution. Store at 4 °C for 4-5 months after reconstitution. Then dilute to 2 mmol/L standard top solution by adding 0.1 mL stock solution into 0.9 mL distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 2/1/0.5/0.25/0.125/0.0625/0.0312 mmol/L.

Substrate: Add 4 mL distilled water to dissolve before use, mix, heat in boiling water bath for 1 minute. Prepare for immediate use or store at 4 °C for 1-2 days.

Positive Control: Briefly centrifuge prior to opening. Add 0.2 mL distilled water to dissolve before use, mix. Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. Sample Preparation

1. For cell and bacteria samples Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mL Assay Buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s ,repeat 30 times); centrifuged at 8000g 4°C for 10 minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples Weigh out 0.1 g tissue, homogenize with 1 mL Assay Buffer on ice, centrifuged at 8000 g 4°C for 10 minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples Detect directly.

6. Assay Procedure

Add following reagents in the microplate :

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Reaction Buffer	50 µl	50 µl	--	--	50 µl
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	--	--
Positive Control	--	--	--	--	10 µl
Substrate	40 µl	40 µl	--	--	40 µl
Mix, put it in the oven, 40°C for 10 minutes.					
Standard	--	--	100 µl	--	--
Distilled water	--	--	--	100 µl	--
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, put it into the convection oven,90 °Cfor 10 minutes, record absorbance measured at 540nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

7. Calculation

Unit Definition:

One unit of β -Amylase activity is defined as the enzyme generates 1 μmol of reducing sugar per minute .

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{Standard}}{V_{Sample}} \times n \ (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \ (U/10^4)$$

2.2 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay}) \times T} \ (U/g)$$

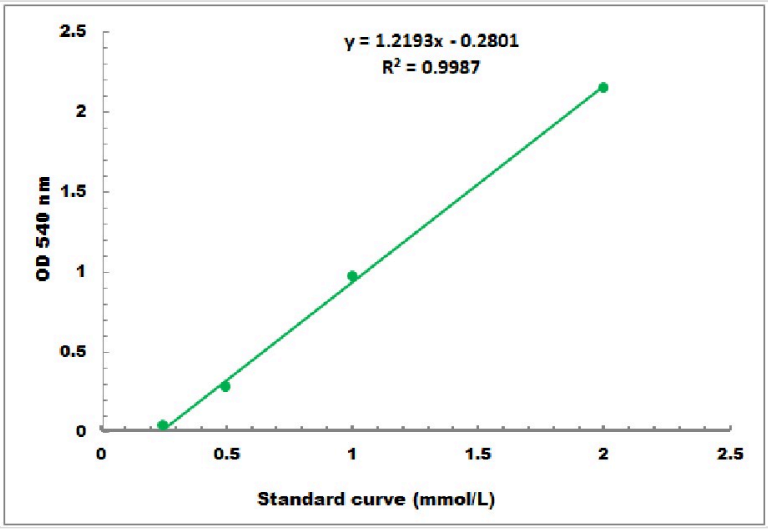
2.3 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \ (U/mL)$$

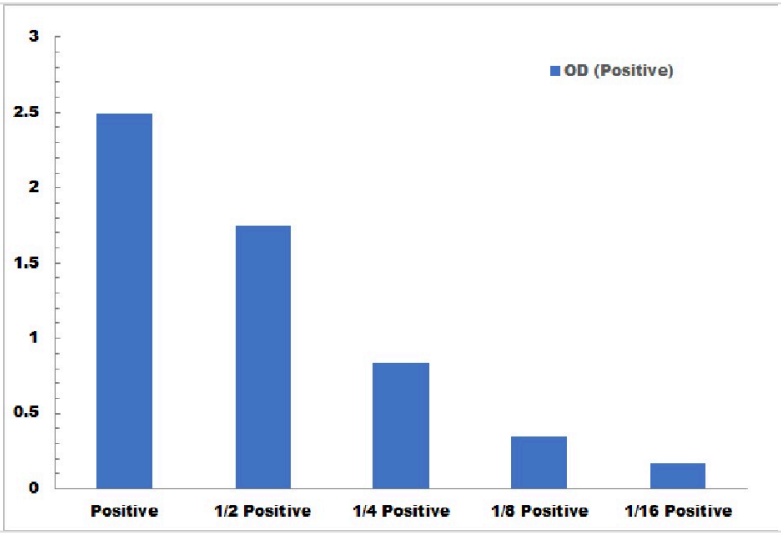
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, mmol/L = μmol/mL
$V_{Standard}$	the volume of standard in assay procedure, mL
V_{Sample}	the volume of sample in assay procedure, mL
V_{Assay}	the volume of Assay Buffer in sample preparation , mL
T	the reaction time , minute

8. Typical Data

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range : 0.2 mmol/L - 2 mmol/L



Positive Control reaction in 96 -well plate assay with decreasing the concentration